Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 2

Amendments to the specification:

Please replace the paragraph at page 54, lines 12-28, with

the following:

Polypeptides of the invention may be post-translationally
modified. Post-translational modifications include
phosphorylation of amino acid residues serine, threonine and/or
tyrosine, N-linked and/or O-linked glycosylation, methylation,
acetylation, prenylation, methylation, acetylation, arginylation,
ubiquination and racemization. One may determine whether a
polypeptide of the invention is likely to be post-translationally
modified by analyzing the sequence of the polypeptide to
determine if there are peptide motifs indicative of sites for
post-translational modification. There are a number of computer
programs that permit prediction of post-translational
modifications. See, e.g., ~~www.expasy.org~~ expasy.org of the world
wide web (accessed August 31, 2001), which includes PSORT, for
prediction of protein sorting signals and localization sites,
SignalP, for prediction of signal peptide cleavage sites,
MITOPROT and Predotar, for prediction of mitochondrial targeting
sequences, NetOGlyc, for prediction of type O-glycosylation sites
in mammalian proteins, big-PI Predictor and DGPI, for prediction
of prenylation-anchor and cleavage sites, and NetPhos, for
prediction of Ser, Thr and Tyr phosphorylation sites in
eukaryotic proteins. Other computer programs, such as those
included in GCG, also may be used to determine post-translational
modification peptide motifs.

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 3

Please replace the paragraph at page 54, lines 29 through page

55, line 8, with the following:

General examples of types of post-translational
modifications may be found in web sites such as the Delta Mass
database ~~http://www.abrf.org/ABRF/Research~~
~~Committees/deltamass/deltamass.html~~ abrf.org/ABRF/Research
Committees/deltamass/deltamass.html of the world wide
web(accessed October 19, 2001); "GlycoSuiteDB: a new curated
relational database of glycoprotein glycan structures and their
biological sources" Cooper et al. Nucleic Acids Res. 29; 332-335
(2001) and ~~http://www.glycosuite.com/~~ glycosuite.com/ of the
world wide web (accessed October 19, 2001); "O-GLYCBASE version
4.0: a revised database of O-glycosylated proteins" Gupta et al.
Nucleic Acids Research, 27: 370-372 (1999) and
~~http://www.cbs.dtu.dk/databases/OGLYCBASE/~~
http://www.cbs.dtu.dk/databases/OGLYCBASE/ of the world wide
web(accessed October 19, 2001);   "PhosphoBase, a database of
phosphorylation sites: release 2.0.", Kreegipuu et al. Nucleic
Acids Res 27(1):237-239 (1999) and ~~http://www.cbs.dtu.dk/~~
~~databases/PhosphoBase/~~ cbs.dtu.dk/ databases/PhosphoBase/ of the
world wide web(accessed October 19, 2001); or
~~http://pir.georgetown.edu/ pirwww/search/textresid.html~~
pir.georgetown.edu/ pirwww/search/textresid.html of the world
wide web (accessed October 19, 2001).

Please replace the paragraph at page 57, lines 1 through 22, with

the following:

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 4

In another embodiment, the invention provides polypeptides that have been post-translationally modified. In one embodiment, polypeptides may be modified enzymatically or chemically, by addition or removal of a post-translational modification. For example, a polypeptide may be glycosylated or deglycosylated enzymatically. Similarly, polypeptides may be phosphorylated using a purified kinase, such as a MAP kinase (e.g, p38, ERK, or JNK) or a tyrosine kinase (e.g., Src or erbB2). A polypeptide may also be modified through synthetic chemistry. Alternatively, one may isolate the polypeptide of interest from a cell or tissue that expresses the polypeptide with the desired post-translational modification. In another embodiment, a nucleic acid molecule encoding the polypeptide of interest is introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide in the desired fashion. If the polypeptide does not contain a motif for a desired post-translational modification, one may alter the post-translational modification by mutating the nucleic acid sequence of a nucleic acid molecule encoding the polypeptide so that it contains a site for the desired post-translational modification. Amino acid sequences that may be post-translationally modified are known in the art. See, e.g., the programs described above on the website ~~www.expasy.org~~ expasy.org of the world wide web. The nucleic acid molecule is then be introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide. Similarly, one may delete sites that are post-translationally modified by either mutating the nucleic acid sequence so that the encoded polypeptide does not contain the post-translational

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 5

modification motif, or by introducing the native nucleic acid molecule into a host cell that is not capable of post-translationally modifying the encoded polypeptide.

Please replace the paragraph at page 59, line 29 through page 60, line 10, with the following:

Plasmid vectors will typically be introduced into chemically competent or electrocompetent bacterial cells. E. coli cells can be rendered chemically competent by treatment, e.g., with $CaCl_2$, or a solution of Mg^{2+}, Mn^{2+}, Ca^{2+}, Rb^+ or K^+, dimethyl sulfoxide, dithiothreitol, and hexamine cobalt (III), Hanahan, J. Mol. Biol. 166(4):557-80 (1983), and vectors introduced by heat shock. A wide variety of chemically competent strains are also available commercially (e.g., Epicurian Coli® XL10-Gold® Ultracompetent Cells (Stratagene, La Jolla, CA, USA); DH5α competent cells (Clontech Laboratories, Palo Alto, CA, USA); and TOP10 Chemically Competent E. coli Kit (Invitrogen, Carlsbad, CA, USA)). Bacterial cells can be rendered electrocompetent, that is, competent to take up exogenous DNA by electroporation, by various pre-pulse treatments; vectors are introduced by electroporation followed by subsequent outgrowth in selected media. An extensive series of protocols is provided online in Electroprotocols (BioRad, Richmond, CA, USA) (http://www.biorad.com/LifeScience/pdf/ New_Gene_Pulser.pdf biorad.com/LifeScience/pdf/_New_Gene_Pulser.pdf of the world wide web).

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 6

Please replace the paragraph at page 61, lines 1 through 18, with the following:

Mammalian and insect cells can be directly infected by packaged viral vectors, or transfected by chemical or electrical means. For chemical transfection, DNA can be coprecipitated with CaPO$_4$ or introduced using liposomal and nonliposomal lipid-based agents. Commercial kits are available for CaPO$_4$ transfection (CalPhos™ Mammalian Transfection Kit, Clontech Laboratories, Palo Alto, CA, USA), and lipid-mediated transfection can be practiced using commercial reagents, such as LIPOFECTAMINE™ 2000, LIPOFECTAMINE™ Reagent, CELLFECTIN® Reagent, and LIPOFECTIN® Reagent (Invitrogen, Carlsbad, CA, USA), DOTAP Liposomal Transfection Reagent, FuGENE 6, X-tremeGENE Q2, DOSPER, (Roche Molecular Biochemicals, Indianapolis, IN USA), Effectene™, PolyFect®, Superfect® (Qiagen, Inc., Valencia, CA, USA). Protocols for electroporating mammalian cells can be found online in Electroprotocols (Bio-Rad, Richmond, CA, USA) ((http://www.bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf see biorad.com/LifeScience/pdf/New_Gene_Pulser.pdf on the world wide web); Norton et al. (eds.), Gene Transfer Methods: Introducing DNA into Living Cells and Organisms, BioTechniques Books, Eaton Publishing Co. (2000); incorporated herein by reference in its entirety. Other transfection techniques include transfection by particle bombardment and microinjection. See, e.g., Cheng et al., Proc. Natl. Acad. Sci. USA 90(10): 4455-9 (1993); Yang et al., Proc. Natl. Acad. Sci. USA 87(24): 9568-72 (1990).

Please insert the following paragraphs at page 118, line 11:

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 7

Example 1a: Suppression subtractive hybridization (Clontech PCR-SELECT)

Clontech PCR-SELECT is a PCR based subtractive hybridization
method designed to selectively enrich for cDNAs corresponding to
mRNAs differentially expressed between two mRNA populations
(Diatchenko et al, *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 6025-
6030, 1996).

Clontech PCR-SELECT is a method for enrichment of
differentially expressed mRNAs based on a selective
amplification. cDNA is prepared from the two mRNA populations
which are to be compared (Tester: cDNA population in which the
differentially expressed messages are sought and Driver: cDNA
population in which the differentially expressed transcripts are
absent or low). The tester sample is separated in two parts and
different PCR adapters are ligated to the 5' ends. Each tester
is separately annealed to excess driver (first annealing) and
then pooled and again annealed (second annealing) to excess
driver. During the first annealing, sequences common to both
populations anneal. Additionally the concentration of high and
low abundance messages are normalized since annealing is faster
for abundant molecules due to the second order kinetics of
hybridization. During the second annealing cDNAs unique or
overabundant to the tester can anneal together. Such molecules
have different adapters at their ends. The addition of
additional driver during the second annealing enhances the
enrichment of the desired differentially expressed sequences.
During subsequent PCR, molecules that have different adapters at
each end amplify exponentially. Molecules which have identical
adapters, or adapters at only one end, or no adapters (driver

Attorney Docket No.:   DEX-0271
Inventors: Macina et al.
Serial No.:   10/001,883
Filing Date: November 20, 2001
Page 8

sequences) either do not amplify or undergo linear amplification.
The end result is enrichment for cDNAs corresponding to
differentially expressed messages (unique to the tester or
upregulated in the tester). This technique was used to identify
transcripts unique to colon tissues or messages overexpressed in
colon cancer tissues.

Pairs of matched samples were isolated from the same
patient, a cancer sample, and the "normal" adjacent tissue from
the same tissue type. The mRNA from the cancer tissue was used as
the "tester", and the non-cancer mRNA as a "driver". The non-
cancer "driver" is from the same individual and tissue as the
cancer sample (Matched). Alternatively, the "driver" can be from
a different individual but the same tissue as the tumor sample
(unmatched). In some cases "driver" mixtures of mRNAs derived
from non-cancer tissues types different from the cancer tissue
type are used. The last approach allows the identification of
transcripts whose expression is specific or upregulated in the
cancer tissue type analyzed. Such transcripts may or may not be
cancer specific in their expression.

Several subtracted libraries were generated for colon
tissue. The product of the subtraction experiments was used to
generate cDNA libraries. These cDNA libraries contain Expressed
Sequence Tags (ESTs) from genes that are colon specific, or
upregulated in colon tissue. Randomized clones picked from each
cDNA PCR Select library were sequenced and the genes identified
by a systematic analysis of the sequence data against the LIFESEQ
Gold database available from Incyte Pharmaceuticals, Palo Alto.
The sequences with no significant homology to any DNA sequence
present in the database are considered novel sequences. These

Attorney Docket No.:   **DEX-0271**
Inventors: **Macina et al.**
Serial No.:   **10/001,883**
Filing Date: **November 20, 2001**
Page 9

colon cancer specific markers are DEX0122_1 through DEX0122_70
and correspond to markers of the instant sequence listing as
taught herein at pages 126-128.